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ORIGINAL CONTRIBUTION

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Effect of dietary canola oil and its degree of oxidation on exocrine pancreatic secretions in growing pigs

Untersuchungen am Modelltier Schwein zum Einfluß von unterschiedlich oxidiertem Rapsöl auf die exokrine Pankreassekretion

Summary Four barrows, average initial weight 35 kg, were fitted with permanent pancreatic re-entrant cannulas and used to determine the effect of level and quality of dietary fat on exocrine pancreatic secretions. The pigs were fed four corn starch-based diets that contained 15 % crude protein from isolated soy protein. Diet 1 contained no canola oil (C-0); diet 2, 15 % canola oil (C-15); diet 3, 15 % canola oil that was heated under vacuum at 180° C for 12 h (C-15/12); diet 4, 15 % canola oil that was heated under vacuum at 180° C for 24 h (C-15/24). Heat treatment resulted in a 4- to 5-fold increase in the content of malonaldehyde which is derived from the oxidation of fatty acids and which is closely related to odour and rancidity in lipids. The experiment was carried out according to a 4 x 4 Latin square design. The pigs were fed twice daily, at 08:00 and 20:00 h, 900 g each meal. Following an adaptation period of 7 d, pancreatic juice was collected continuously for 24 h at 2-h intervals from 08:00 on d 8 until 08:00 on d 9 and from 08:00 on d 10 until 08:00 on d 11 during each experimental period. The volume of secretion of pancreatic juice peaked 6–10 h postprandially and was similar ($P > .05$) during day (08:00–20:00 h) and night (20:00–08:00 h). Replacement of 15 % starch by 15 % canola oil resulted in a decrease ($P < .05$) in the secretion of α-amylase and an increase ($P < .05$) in the secretion of lipase. The inclusion of oxidized fat caused a further increase ($P < .05$) in total lipase activities. It can be concluded that the exocrine pancreas is able to adapt to variations in the level and quality of dietary lipids.

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Zusammenfassung Vier Börge mit einem mittleren Anfangsgewicht von 35 kg wurden mit permanenten Pankreas-Umleitungskanülen versehen, um den Einfluß von Fettmenge und -qualität auf die exokrine Pankreassekretion zu untersuchen. Die Tiere erhielten synthetische Rationen auf der Grundlage von Maisstärke und Sojaproteinisolat mit einem Rohproteingehalt von 15 %. Ration C-0 enthielt kein Rapsöl, während die übrigen drei Rationen 15 % Rapsöl im Austausch gegen Maisstärke enthielten, und zwar frisches Rapsöl (Ration C-15) oder unter Vakuum bei 180° C über einen Zeitraum von 12 h (Ration C-15/12) bzw. 24 h (Ration C-15/24) erhitzt. Die Versuchsanordnung entsprach einem 4 x 4 Lateinischen Quadrat. Die Diäten wurden in zwei Mahlzeiten (900 g um 08:00 und 20:00 h) verabreicht. Nach einer Adaptationsphase von 7 d wurde das Pankreassekret kontinuierlich in 2-h-Intervallen in zwei zeitlich voneinander getrennten Sammelperioden über jeweils 24 h quantitativ erfaßt. Das Maximum der Volumensekretion ergab sich 6–10 h postprandial; die Volumensekretion in der Tag- (08:00–20:00 h) und Nachtphase (20:00–08:00 h) zeigte keine signifikanten ($P > .05$) Unterschiede. Die Substitution von 15 % Stärke durch 15 % Rapsöl führte zu einer deutlichen ($P < .05$) Abnahme der α-Amylase zugunsten einer erhöhten ($P < .05$) Lipasesekretion. Die Fettoxidation durch Erhitzen verursachte einen weiteren Anstieg ($P < .05$) der Lipaseausscheidung. Die Untersuchungen belegen, daß die exokrine Pan-

kreassekretion beim Schwein durch uantitative und qualitative Veränderungen des Nahrungsfettes zu beeinflussen ist.

Key words Pig – fat – pancreatic secretions – enzymes

Schlüsselwörter Schwein – Fett – Pankreassekretion – Enzyme

Introduction

The pancreatic exocrine secretions must be considered the most potent digestive secretions since its enzymatic equipment contributes to the hydrolysis of the major dietary nutrients such as proteins, carbohydrates and lipids. Furthermore, the pancreas can adapt to changes in the dietary composition by altering the rate of biosynthesis of enzymes involved in the enzymatic breakdown of various dietary nutrients. The mechanisms of pancreatic adaptation to the diet have been reviewed by Corring (5, 6) and more recently, by Corring et al. (8) and Makkink and Verstegen (16).

However, there is a scarcity of information on the effect of dietary fat and, in particular, its quality on the secretory activity of the pancreas in pigs. For example, hydroperoxides, which are the primary products from the oxidation of unsaturated fatty acids during storage and processing of fat, are involved in the development of rancidity, the production of odours and flavours and the formation of toxic and physiologically active compounds. In addition, in vitro studies have demonstrated interactions of lipid peroxides and their secondary degradation products with protein including a decrease in enzyme activity, loss of protein solubility and destruction of specific amino acids (17).

The objective of the present study was to examine the effect of level and quality of dietary fat on exocrine pancreatic secretions in pigs fitted with permanent pancreatic re-entrant cannulas, which allow for long-term continuous collection of pancreatic juice.

Materials and methods

Four barrows (Yorkshire x Lacombe), with an average initial weight of 35 kg, were obtained from the University of Alberta swine herd. The pigs were housed in stainless steel metabolism crates 1 week prior to surgery and fed a 16 % crude protein grower diet (26). Water was freely available from a low-pressure drinking nipple.

A surgical procedure was previously developed for pigs that allows for long-term collection, sampling and subsequent return of pancreatic juice (12). A re-entrant cannula prepared from Plastisol (F.H. and Sons Manufacturing Ltd., Concord, Ontario, Canada) is used to conduct pancreatic juice from a small isolated segment of the duodenum (referred to as the pouch), which receives the pancreatic duct, through permanent intercostal fistulas, back into the duodenum, which is reconnected by end-to-end anastomosis within 3 cm of the normal entry point of the pancreatic duct. A schematic presentation of this technique is presented in figure 1. This procedure, also referred to as the "Pouch Method", allows for collection of pancreatic juice for up to 4 to 5 months, at times longer, in contrast to the direct cannulation method by which pancreatic juice can be collected for only 1 to 2 months following surgery (7). A more detailed description of pre- and post-operative care and of the construction of the re-entrant cannula was given by Hee et al. (12).

Following a 3-week recuperation period, during which time the barrows were fed the grower diet, the pigs received four corn starch-based diets that contained 15 % crude protein from isolated soy protein (Table 1). Diet 1

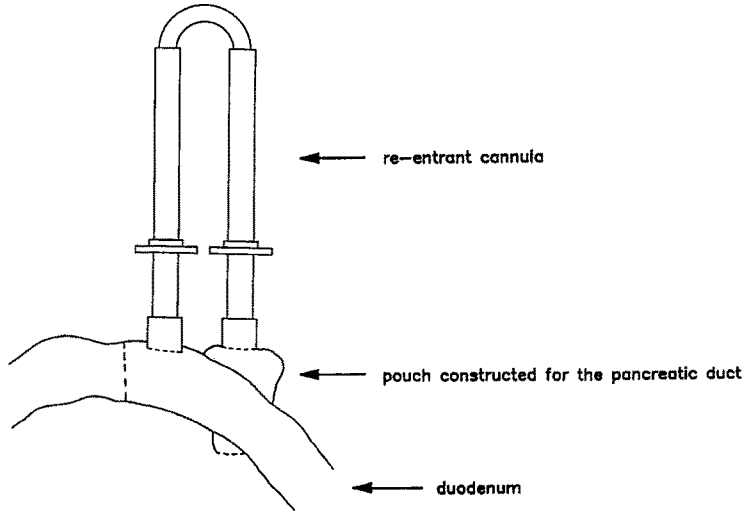

Fig. 1 Placement of pancreatic cannula

Table 1 Formulation of the experimental diets and content of malonaldehyde

Diets[a]	C-0	C-15	C-15/12	C-15/24
Ingredients, %				
Soy protein isolate	17.2	17.2	17.2	17.2
Corn starch	64.4	49.4	49.4	49.4
Canola oil	–	15.0	15.0	15.0
Dextrose	10.0	10.0	10.0	10.0
Alphafloc[b]	5.0	5.0	5.0	5.0
Trace-mineralized salt[c]	.5	.5	.5	.5
Vitamin-mineral premix[d]	2.9	2.9	2.9	2.9
Malonaldehyde, mg/kg	.4	3.2	13.8	16.7

[a] C-0: without canola oil; C-15: 15 % unheated canola oil; C 15/12: 15 % canola oil heated under vacuum at 180 °C for 12 h; C 15/24: 15 % canola oil heated under vacuum at 180 °C for 24 h

[b] Brown Company, Berlin, N.H., USA

[c] Windsor Salt Co. (Toronto, Ontario, Canada). Composition (mg/g): NaCl, 965; ZnO, 4; $FeCO_3$, 1.6; MnO, 1.2; CuO, .33; $Ca(IO_3)_2$, .07; CaO, .04

[d] The vitamin and mineral mixtures provided the following (mg/kg diet): retinyl palmitate, 5.2; cholecalciferol, .375; all-*rac*-α-tocopherol acetate, 44; menadione, 2; riboflavin, 2.2; niacin, 12; panthothenic acid, 11.2; vitamin B_{12}, .011; choline, 550; thiamin, 1.1; pyridoxine, 1.1; biotin, .1; folic acid, .6; Fe, 50; Zn, 50; Mn, 2; Cu, 3; I, .14; Se, .15

contained no canola oil (C-0); diet 2, 15 % canola oil (C-15); diet 3, 15 % canola oil that was heated under vacuum at 180° C for 12 h (C-15/12); diet 4, 15 % canola oil that was heated under vacuum at 180° C for 24 h (C-15/24). Canola oil was included at the expense of corn starch. The remainder of the diets was made up of 10 % dextrose to possibly improve the palatability of these purified diets and 5 % Alphafloc to provide a source of fiber. Trace-mineralized salt (.5 %) and a vitamin-mineral premix (2.9 %) supplied vitamins and minerals according to NRC (19) standards.

The pigs were fed equal amounts of the diets, 900 g each meal at 08:00 and 20:00 h. The experiment was designed in the form of a 4 x 4 Latin square design. Each experimental period comprised 14 d. Following an adaptation period of 7 d, pancreatic juice was collected continuously for 24 h at 2-h intervals from 08:00 on d 8 until 08:00 h on d 9 and from 08:00 on d 10 until 08:00 h on d 11. After the collection was completed, the pigs were allowed a 3-d recuperation period before they were switched to the next experimental period. The collection, sampling and subsequent return of pancreatic juice were carried out according to Hee et al. (13). Samples were immediately frozen at -20° C after collection.

Chemical and statistical analyses

Samples of pancreatic juice (from 2-h samples) were pooled per experimental diet to determine the pattern of secretion of volume of pancreatic juice during both 24-h periods. Samples were also pooled within pigs and experimental diets to determine the effect of diets on the daily pattern of volume of secretion of pancreatic juice, total protein and enzymes.

Measurement of malonaldehyde

The quantitative determination of malonaldehyde in the diets was performed using a distillation method described by Tarladgis et al. (29).

Measurement of protein

Protein in pancreatic juice was determined according to the method of Lowry et al. (15) using bovine serum albumen as standard.

Measurement of trypsin and chymotrypsin activities

Pancreatic juice was diluted 10-fold with a buffer solution at 5 °C and the proteolytic enzymes were activated with an equal amount of a saturated solution of enterokinase (enteropeptidase; EC 3.4.21.9). The enterokinase (5 units/mg) was isolated from porcine intestine as a partially purified salt-free lyophilized powder containing approximately 65 % protein (Sigma Chemical Co., St. Louis, MO). The enterokinase solution was prepared by dissolving 1 g enterokinase in 100 ml distilled and de-ionized water, centrifuging the solution at 16,300 g for 20 min at 5 °C and decanting the supernatant for immediate use. The buffer solution contained 100 µg bovine serum albumen, 50 mM $CaCl_2$ and 50 mM Tris-HCl per ml (11). The mixtures were incubated at 5 °C for 5 and 3 h for trypsin and chymotrypsin, respectively. Trypsin activity was measured at 25 °C and pH 8.1 using α-N-toluene-p-sulphonyl-L-arginine methyl ester (TAME) as substrate

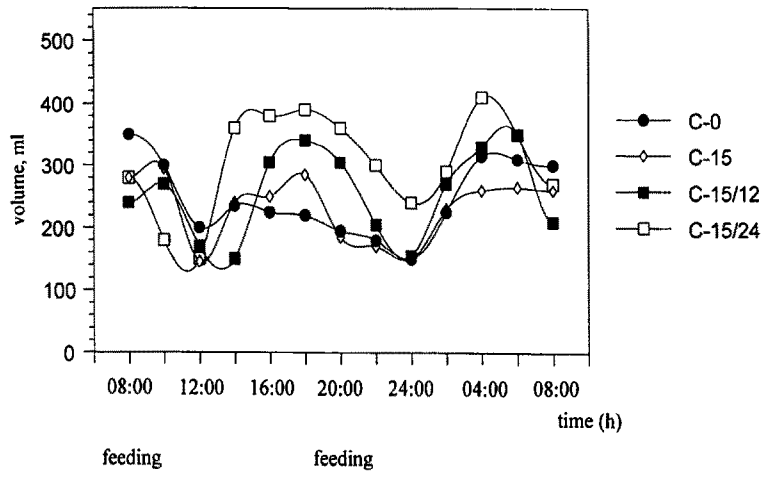

Fig. 2 The pattern of volume of secretion of pancreatic juice in pigs fed different diets during day (08:00–20:00 h) and night (20:00–08:00 h)

(22). Chymotrypsin activity was measured at 25 °C and pH 7.8 using N-benzoyl-L-tyrosine ethyl ester (BTEE) as substrate (23). A spectrophotometer (Gilford 2600, Gilbert Instrument Laboratories, Ohio 44074) was used to measure the extinction changes.

Measurement of a-amylase activity

Pancreatic juice was diluted 200-fold with saline at 5 °C. The amylase activity was measured at 25 °C and pH 6.9 using a solution of soluble potato starch (Linter's starch) as substrate according to procedures described by Rick and Stegbauer (24).

Measurement of lipase activity

Pancreatic juice was diluted 2500-fold with distilled and de-ionized water. The lipase activity was measured at 30 °C and pH 8.5 using a gum arabic stabilized emulsion of triolein (olive oil suspension) as substrate according to procedures described by Schmidt et al. (27).

Specific activity of enzymes secreted into pancreatic juice is expressed as units (U)/l. One U of enzyme activity is defined as the hydrolysis of 1 µmol substrate in 1 min. Total enzyme activities were calculated as follows: specific activity x volume of pancreatic juice.

Analysis of variance to compare pancreatic secretions between diets was performed according to procedures described by Steel and Torrie (28). Where appropriate, treatment means were compared using the Student-Newman-Keuls multiple range test. Treatment means were considered statistically significant at $p < .05$.

Results and discussion

The pigs remained healthy and consumed their daily meal allowances of the experimental diets throughout the dur-

Table 2 The effect of diet composition on the daily volume of secretion of pancreatic juice, protein and total enzyme activities in pancreatic juice of pigs fed the experimental diets

	Diet							
	C-0		C-15		C-15/12		C-15/24	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Volume, ml/24 h	2954	714	2788	756	3121	1243	3690	194
Protein, g/24 h	17.3[a]	4.8	19.9[a, b]	5.7	22.5[a, b]	3.9	22.0[b]	1.0
Total enzyme activity, U/24 h x 10^{-3}								
Trypsin	176.1	68.0	218.3	26.0	228.4	28.0	241.6	106.0
Chymotrypsin	117.0[a,b]	13.2	107.4[a]	8.4	138.0[b]	4.7	128.3[a,b]	37.4
α-amylase	356.2[a]	101.4	177.8[b]	32.0	146.3[b]	46.3	262.5[c]	31.9
Lipase	29.6[a]	10.7	101.7[b]	13.5	250.0[c]	25.8	181.1[c]	55.5

[a,b,c] Means in the same row with different superscripts differ at $p < .05$

ation of the investigations. The meal allowances were usually consumed within 10 to 15 min after feeding. Post-mortem examinations were carried out at the conclusion of the experiment and revealed no intestinal adhesions. In addition, there was no dilatation of the pouch and the mucosa showed no signs of irritation or damage. Heat treatment under vacuum at 180 °C for 12 and 24 h resulted approximately in a 4- and 5-fold increase in the content of malonaldehyde in diet C-15/12 and C-15/24, respectively, as compared to diet C-15. Tarladgis et al. (29) describe malonaldehyde as a three-carbon fragment which is derived from the oxidation of mono- or polyenoic fatty acids and which is closely related to odour and rancidity in lipids.

In agreement with studies reviewed by Corring (6), the present results show that pancreatic secretions are continuous in the pig. A typical pattern emerged for the hourly changes in the volume of secretion of pancreatic juice with maximum levels occuring at approximately 6–10 h after feeding before decreasing gradually to prefeeding levels (Figure 2). This pattern was more distinct for the diets that contained heated canola oil (C-15/12 and C-15/24) than for the diets without canola oil (C-0) or 15 % unheated canola oil (C-15) but is in general agreement with previous studies in which pigs were also fed twice daily at 12-h intervals (13, 18, 21).

The pattern of hourly changes in pancreatic secretions were similar during day (08:00–20:00 h) and night (20:00–08:00 h) with time after feeding. In addition, the volume of secretion was similar ($p > .05$) between day (08:00–20:00 h) and night (20:00–08:00 h) which has also been reported by Hee et al. (13, 14) and Mosenthin and Sauer (18). As was pointed out by Hee et al. (13, 14) it may be feasible to obtain representative results from a 12-h collection, provided the pigs are fed twice daily equal amounts each meal at 12-h intervals. The stress that is placed on the pig during collection and its possible effect on pancreatic secretions can thereby be minimized. However, no consistent diurnal pattern of pancreatic secretions and no clear response to feeding was obtained when pigs were fed twice daily, 6 h apart, at 09:00 and 15:00 h (20). Hee et al. (14) assume that this feeding regimen per sé, as compared to feeding at 12-h intervals, may be responsible for the absence of a consistent diurnal variation.

The effect of diet on the daily volume of secretion of pancreatic juice and the secretion of protein and total enzyme activities is presented in table 2. Both the daily flow of pancreatic juice and the total daily protein secretion were higher in diets C-15/12 and C-15/24 than in diets C-0 and C-15. The increase in protein secretion can be attributed to the combined increases in total enzyme activities. However, due to the large variation within treatments, these differences were only significant for daily protein secretions between diet C-0 (no canola oil) and diet C-15/24 (15 % oxidized canola oil). As was reviewed by Makkink and Verstegen (16) values for the daily volume of secretion of pancreatic juice and the secretion of protein are quite variable ranging from 1.0 to 5.0 l and from 4.7 to 28.9 g, respectively, for pigs in the same weight range as reported in this study. A direct comparison of results, as opposed to relative differences resulting from various dietary treatments, is difficult because these comparisons are confounded by differences in feed intake, feeding regimen, diet composition, body weight, different methods to collect pancreatic juice and analysis of enzyme activities.

With regard to trypsin and chymotrypsin the total activities of these proteolytic enzymes were largely unaffected by the dietary treatments. The trend towards higher total activities of trypsin and chymotrypsin in pigs fed diets containing higher levels of oxidized canola oil (diet C-15/12 and diet C-15/24) corresponds to the higher daily protein secretion in pancreatic juice of these pigs. Despite the fact that the daily secretion of chymotrypsin was higher ($P < .05$) in diet C-15/12 than in diet C-15, which can be mainly attributed to an extremely small variation within these treatments, adaptative responses of the proteolytic enzymes to dietary changes were unlikely to occur since these diets did not differ in protein content and composition.

Corring (4), in studies with the direct pancreatic duct cannulation technique and Hee et al. (13) with the "Pouch Method", showed a direct relationship between the dietary starch and fat content and the secretions of α-amylase and lipase, respectively. A similar relationship was observed in the present studies with the "Pouch Method". The replacement of 15 % starch by 15 % fresh canola oil (diet C-15) increased ($P < .05$) the total secretion of lipase by approximately 360 %. Corring (4) and Hee et al. (13) reported an increase in the level of lipase in pancreatic juice by approximately 230 and 600 % when the dietary fat content was increased from 3 to 21 % and from 2 to 10 %, respectively. As can be derived from studies by Corring (4) the levels of α-amylase and lipase in pancreatic juice varied in the same direction but not proportionally when the dietary starch content was reduced from 60 to 20 % and the dietary fat content was increased from 3 to 21 %. This is in general agreement with the results of the present study.

Pancreatic lipase adaptation to dietary lipids has also been reported in other species including the rat (2, 9), dog (1) and poultry (30).

However, it appears that there are minimal and maximal limits in the secretion of lipase in response to differences in the dietary supply of lipids. In studies with rats, Sabb et al. (25) demonstrated that lipase activity is not stimulated when energy from fat supplies 196 KJ or less of the total dietary energy intake; beyond this point, lipase activity increases and peaks when energy from fat supplies 225–280 KJ of the total dietary energy. Similarly, Bucko and Kopec (3) reported that the maximal

pancreatic response to corn oil is obtained with 18 % oil in the diet. Gidez (10) assumes that the relation between dietary carbohydrates and lipids, in terms of assimilable energy, would explain why variations in lipase activity are either non existent or high. These results and conclusions, however, have not been confirmed yet in studies with pigs.

The results obtained in this study provide evidence that besides the quantity of fat in the diet the quality of fat may affect the secretion of lipase in pancreatic juice. The oxidation of fatty acids by different heat treatments, which resulted in higher levels of malonaldehyde in diets C-15/12 and C-15/24, caused an increase ($P < .05$) in total lipase activities compared to diets C-0 and C-15. In addition, the daily secretion of α-amylase was higher ($P < .05$) in diet C-15/24 than in diet C-15/12. However, at present, there is no explanation for this difference; this enigma provides an interesting challenge for future research.

Only very few studies have been carried out so far that focus on the effect of differences in fat quality on pancreatic secretions. According to Corring (6) the specific lipase activity in pancreatic juice of pigs increases by 700 % when the triglyceride intake is increased from 30 to 320 g. Studies in rats reveal that lipase biosynthesis is stimulated more by unsaturated than saturated fatty acids (9). According to Sabb et al. (25) the secretion of pancreatic lipase adapts primarily to the amount of dietary fat and is affected by the type of fat only below the level of maximal response.

In conclusion, this study provides evidence that the secretion of pancreatic α-amylase and lipase is very sensitive to changes in the dietary content of starch and lipids, respectively. Furthermore, it can be derived from the results of this study that oxidation of fatty acids stimulates the biosynthesis of pancreatic lipase. However, mechanisms underlying pancreatic responses to changes in fat quality have been little investigated and, thus, remain poorly understood.

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